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COMPLEMENT FIXATION IN TYPHOID FEVER

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In the last few decades, with the steady advancement of our serologic and bacteriologic knowledge of typhoid fever, new and efficient means of diagnosing the disease have been put at our command. But of all such tests only those that combined simplicity with specificity survived. For these reasons the Gruber-Widal reaction was adopted by health centers throughout the civilized world as indispensable for the diagnosis of typhoid fever, although, as a reaction, it is not the earliest immune manifestatation nor is its course uniform.

The field of complement-fixation tests for typhoid is one of the least explored. When one stops to think that typhoid fever was one of the first diseases in which the phenomenon of complement deviation reaction was demonstrated by Bordet and Gengou[1] in 1901, one wonders why so few have taken up the subject later.

The reports of recent investigators on complement fixation in typhoid are conflicting. Rodet and Faber[2] were unable to demonstrate complement fixing bodies in animals immunized against typhoid, while Kolmer,[3] working similarly, obtained positive results. The complexity of reaction, the effect of heating on the immune bodies of serum and the indiscriminate choice of antigen are, no doubt, the main causes for such conflicting reports.

We have lately developed a simple differential quantitative technic for active serum complement fixation which has been applied successfully to the diagnosis of syphilis, gonorrhea and to a limited degree of tuberculosis.[4] The successful application of the same technic to typhoid is described.

TECHNIC

Human serum is used while fresh; it should not be over 48 hours old. After keeping the serum together with the clot for 24 hours it is separated by centrifugalization and distributed equally in 6 pairs of small test tubes in doses of 0.01, 0.02, 0.03, 0.04, 0.05 and 0.10 c c,

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[1] Ann. de l'Inst. Pasteur, 1901, 3, p. 289.

[2] Phys. et Path. Gèn., 1912, 14, p. 86.

[3] Jour. Med. Research, 1912, 21, p. 373.

[4] In press.

respectively. The left hand series of tubes receive 0.5 c c of salt solution each and serve as controls for the hemolytic value of the serum in question. The right hand tubes receive 0.5 c c of the bacillary antigen each. The tubes are placed at 37 C. for 30 minutes and then 0.5 c c of one half % suspension of sensitized sheep cells are added to all. Readings are taken in 15 to 30 minutes or as soon as the complementary value shows a unit of hemolysis in 0.02 or 0.03 c c of serum.

Certain serums are naturally deficient in hemolytic value. In such cases the serum in question is mixed with an equal amount of a typhoid-negative serum of high hemolytic value and the test carried on as usual. The ratio of the hemolytic values in the two series of tubes indicates the degree of specificity in terms of fixed complement units. The whole process does not take more than two hours from the time the specimen of blood is received. Table 1 gives actual duplicates of the readings of a few of our cases as given in table 2.

TABLE 1

TYPHOID COMPLEMENT-FIXATION READINGS OF SOME CASES OF TABLE 2 *

Case	Amount of Serum in C c						Specific Ratio	Plus Equivalent
	0.10	0.05	0.04	0.03	0.02	0.01		
15	C/C	C/3	C/3	C/3	C/3	1/3	0.060 : 0.015	++++
16	C/C	C/1	C/3	C/3	C/3	1/3	0.045 : 0.015	+++
19	C/C	C/3	C/3	C/3	2/3	3/3	0.050 : 0.025	++
26	C/C	C/C	C/1	C/2	C/3	2/3	0.020 : 0.020	+
27	C/C	C/C	C/C	C/C	1/2	2/3	....	Negative

* C indicates complete hemolysis; 3, complete inhibition of hemolysis. Numerals 1 and 2 indicate roughly ⅓ and ⅔ inhibition. Of the two readings under each amount of serum the first indicates the hemolytic and the second the specific value. For example, in case 16 the hemolytic unit is 0.015 c c and the specific 0.045 c c, the specific quotient being 0.045 : 0.015 equals 3 units of fixation, roughly +++: In case 27 as the specific value does not exceed the hemolytic, the case is considered negative.

The success of the test depends mainly on the proper choice of antigen, and it must be born in mind that organisms belonging to the same species may be differentiated immunologically. As a nucleus for our antigen we collect 8 or 10 typhoid vaccines from various sources. Then we test the maximum and minimum antigenic values of this mixture against 0.02 c c of typhoid immune rabbit serum, the amount of the latter depending on its potency. The antityphoid serum of New York Department of Health usually represents one unit of complement fixing value in 0.01 c c. The next step is to find the optimum antigenic value of the mixture by testing out its maximum nonspecific value against typhoid-negative human serums. For example, if we suppose the maximum antigenic dose, 0.50 c c, to be represented in a dilution of

TABLE 2

WEEKLY BACTERIOLOGIC AND SEROLOGIC RESULTS IN FIFTY CASES OF TYPHOID FEVER

Case		First			Second			Third			Fourth			Fifth			Clinical Notes
		BC	CF	AG	BC	CF	AG	BC	CF	AG	BC	CF	AG	BC	CF	AG	
1	R5	+	+	+	—	+	—	—	+	—	..	..	+	..	..	..	Moderately toxic
2	..	..	..	..	+	..	—	..	..	+	..	..	..	..	+	..	Regular course
3	..	+	..	—	..	..	+	..	+	..	..	..	..	..	..	..	Toxic
4	..	+	+	—	..	..	+	..	..	..	..	..	..	..	..	..	Toxic
5	..	+	+	..	..	..	..	—	+2	+2	..	..	..	..	..	..	Regular course
6	..	..	..	..	+	+	—	..	..	+	..	..	..	..	..	..	Moderately toxic
7	..	..	..	..	..	..	..	+	+	..	..	..	..	..	..	..	Died in coma on admission
8	..	..	..	..	..	..	..	—	+	—2	..	..	±	..	..	..	Regular course
9	R3	+	+	..	+	+2	+	..	..	..	..	..	..	..	..	..	Died, Staph. aureus sepsis
10	R3	+	—	+	—2	±	—	—	+2	..	..	..	..	..	..	..	Moderately toxic
11	..	..	..	..	+	+	+	—	..	+2	..	..	+	..	..	..	Regular course
12	..	+	+	—	..	..	..	..	..	..	..	..	..	..	..	..	Toxic
13	..	..	..	..	—	+	+	..	..	..	..	..	..	..	..	..	Regular course
14	R4	+	+	..	..	..	..	—	+	∓2	..	..	..	..	..	..	Regular course
15	..	..	..	..	—	+	+	..	..	..	..	..	..	..	+	..	Regular course
16	R4	—	+	..	—	—	—	—	+	..	..	..	..	..	..	..	Regular course
17	..	..	..	..	+	+	+	+	..	..	..	..	..	..	..	..	Died, Staph. albus sepsis
18	..	..	..	..	..	..	..	—	+	+	..	..	..	..	..	..	Regular course
19	..	..	..	..	..	+	..	..	..	..	..	..	..	..	..	..	Died, internal hemorrhage
20	..	..	..	..	—	+	—	—	+	+	..	..	..	—	+	+	Moderately toxic
21	..	..	..	..	+	+	+	+	..	..	..	..	..	..	..	..	Regular course
22	..	..	..	..	+	+	+	..	..	..	..	..	..	..	..	..	Regular course
23	R4	..	..	..	—	+2	±	—2	+	..	..	..	..	..	..	..	Regular course
24	..	+	+	+	..	..	..	..	..	..	..	..	..	..	..	..	Moderately toxic
25	..	..	..	..	..	..	..	+	+	+	..	..	..	..	..	..	Regular course
26	..	+	+	+	..	..	+	..	..	..	..	..	..	..	..	..	Regular course
27	..	..	..	..	..	..	..	—	—	—	..	..	..	..	..	..	Regular course
28	..	+	+	+	..	..	..	+	..	..	..	..	..	..	..	..	Moderately toxic
29	..	..	..	..	..	..	..	—	+	—	..	..	..	..	..	..	Regular course
30	..	..	..	..	..	..	..	—	+	—	..	+	..	..	..	..	Regular course
31	..	..	..	..	—	+	..	..	+	—	..	..	..	..	..	..	Regular course
32	..	..	..	..	..	+	+	—	+	..	..	..	..	..	..	..	Regular course
33	..	..	..	..	—	—	—	..	..	..	..	+	+	..	..	..	Regular course
34	R3	..	..	..	—2	—	+	..	..	..	..	..	..	..	..	..	Moderately toxic
35	..	..	..	..	..	..	..	—	+	+2	..	..	..	..	..	..	Regular course
36	..	+	—	+2	..	..	..	..	..	..	..	..	..	..	..	..	Toxic
37	..	..	..	..	..	—	∓	..	..	..	..	..	..	..	..	..	Regular course
38	..	..	..	..	..	..	..	..	+	+	..	..	..	..	..	..	Regular course
39	..	..	..	..	..	—	+2	..	..	..	..	..	..	..	..	..	Regular course
40	..	..	—	—	—	..	±	..	..	—	..	..	..	..	..	..	Moderately toxic
41	..	+	+	—4	+	+	—3	—	..	—	..	..	..	..	..	..	Moderately toxic
42	..	+	+	∓	..	..	..	..	..	..	..	..	..	..	..	..	Moderately toxic
43	..	+	—	+2	..	..	—2	—	..	..	..	..	..	..	..	..	Moderately toxic
44	..	..	..	..	..	..	..	..	+	—	..	..	+	..	..	..	Regular course
45	..	..	..	..	+	+	+	..	..	..	..	..	..	..	..	..	Moderately toxic
46	R4	..	+	—	∓	+	—2	∓	..	+	..	..	..	..	..	..	Moderately toxic
47	..	..	..	..	..	..	..	..	+	∓	—	..	+2	..	..	..	Moderately toxic
48	..	..	—	±	—	—	..	..	..	..	..	..	..	..	..	..	Moderately toxic
49	..	..	—	—	—	..	—	—	—	..	..	..	..	..	..	±	Moderately toxic
50	..	..	..	..	—	+	..	..	..	..	..	..	..	..	..	..	Toxic
Total +..		15	13	11	10	21	18	6	22	17	..	2	7	..	3	2	Blood culture average % 44+
Total —..		1	6	12	17	7	18	20	2	12	1	..	1	1	..	1	Complement fixation average % 80+
% +......		94	68	50	40	75	50	23	92	60	..	100	88	..	100	70	Agglutinin reaction average % 55+

BC, CF, AG, indicate blood culture, complement fixation and agglutination, respectively. R indicates relapses and recrudescences, and the numerals that follow it immediately indicate the week during which they have occurred. The reactions and cultural findings are reported either as positive, + or negative, —. Each sign stands for a single test; consequently ∓ means a negative and positive finding. The numerals following the signs indicate the number of repeated tests during the weekly interval.

1 : 50 and the minimum in a dilution of 1 : 100, by testing out intermediate dilutions between these two values we finally reach the optimum dilution, the last in the descending scale, that will give a clear negative reading as in case 27, table 1.

An antigen prepared and standardized in this way keeps for months in the icebox and answers the average demands. In hospitals, however, where blood cultures are taken in all septic cases, one at times meets cases of typhoid fever with positive blood culture and negative complement fixation. By further enriching the antigen with such rare strains a very potent antigen may be developed capable of detecting at least 80% of cases in practically all stages of the disease, from the first to the fifth week.

TABLE 3

Comparative Clinical Value of Blood Culture, Complement Fixation and Agglutination in the Diagnosis of Typhoid

Type of Reaction			Number of Cases	Percentage
Blood Culture	Complement Fixation	Agglutination		
+	+	+	12	30
—	+	+	9	22
—	+	—	7	18
+	+	—	6	15
—	—	—	3	8
+	—	+	2	5
—	—	+	1	2
Total................	....	....	40	100
50	85	60	Average percentage per reaction	

This technic has been applied to 50 cases of typhoid and over 100 control cases. The complement fixations were controlled by blood cultures and agglutination tests. We were thus able to make a comparative study of the efficiency and specificity of complement fixation and the other two tests in the diagnosis of typhoid fever.

The technic used in agglutination was the dried blood, microscopic, loop method, with two dilutions of serum; 1 : 10 and 1 : 50. Complete agglutination with complete loss of motility in both dilutions is considered positive. On the other hand, complete agglutination in both with partial loss of motility either in one or both dilutions, and vice versa, is considered as doubtful. With clear negative controls, both types of reaction are highly specific, as the average percentage of nonspecific agglutinations, considering even doubtful reactions as positive, was within 8 (table 4). For the blood cultures we used no special mediums. About 10 c c of ordinary nutrient agar were inoculated with 2 c c of blood and 3 or 4 plates poured. Growth, if any, invariably appeared within 3 days; otherwise cultures were considered negative.

The weekly results in the 50 cases of typhoid fever are given in table 2. In table 3 we have separated the cases in which all 3 tests show the degree of superiority of each test in the clinical course of the were either done simultaneously or within the same weekly interval to disease. The resumé of table 2 incorporating also our control cases is given in table 4 and shows the percentage of specific and nonspecific reactions. Finally, in chart 1, we have tried to give a visual presentation of the weekly course of each reaction on a percentage basis.

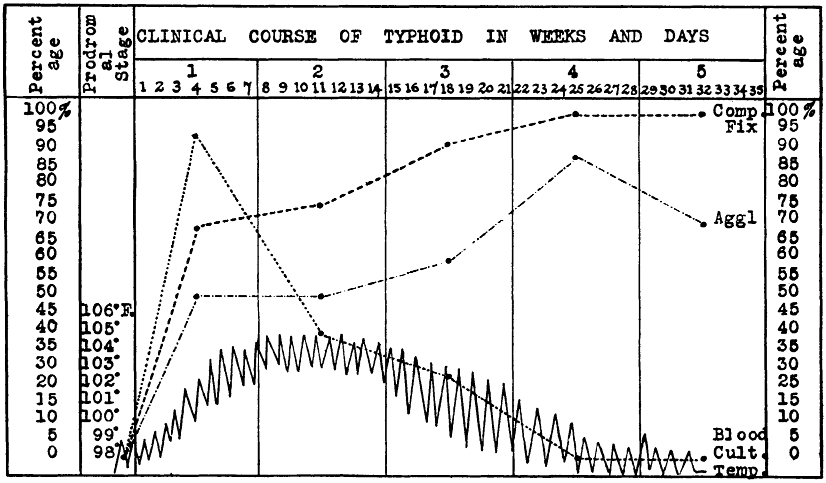

Chart 1.—The weekly course of blood culture and immune reactions in typhoid fever.

The tables and chart emphasize the significance of complement fixation as a diagnostic means of great efficiency in detecting typhoid fever. It is true that during the first week of the disease the blood culture is superior to the immunologic tests; but beginning with the second week, while the blood culture curve falls abruptly, the immune reaction curves rise steadily, the fixation curve always preceding. During the fourth week the agglutinin curve reaches its peak at 88%, thereafter showing a tendency to fall, while the complement fixing curve, from the fourth week and on, stands at 100%. What happens to it after the fifth week we are not in a position to say, as our investigations do not extend beyond the hospital stay of the patients.

As this study was undertaken to investigate primarily the value of complement fixation as a diagnostic means, the question arises whether by using special medium for blood culture and a more sensitive method for agglutination better results might not have been secured. Coleman and Buxton,[5] with their special bile-broth medium for blood cultures in typhoid fever, obtained 69% positives. They also recapitulate the blood culture work in typhoid up to 1917, including the results of 1,602 individual blood cultures by 33 European and American investigators, the average percentage of positives being 75%. The weekly findings of Coleman and Buxton, together with the weekly agglutination reports of Park and Williams,[6] are compared with our findings in table 5.

TABLE 4

TOTAL CASE AND PERCENTAGE RECORDS OF BLOOD CULTURE, COMPLEMENT FIXATION AND AGGLUTINATION IN 50 TYPHOID AND 100 NONTYPHOID CASES INCLUDING NONSPCIFIC REACTIONS

Type of Disease	Type of Reaction	Blood Culture		Complement Fixation		Agglutination	
		Number of Cases	Percentage	Number of Cases	Percentage	Number of Cases	Percentage
Typhoid	Positive	31	45	61	80	55	55
Typhoid	Negative	40	55	15	20	44	45
Not typhoid	Positive	..	..	4	2*	16	8*
Not typhoid	Negative	29	100	120	98	85	92
Total		100	...	200	...	200	

* The percentages of nonspecific reactions were computed on the total number of tests, including typhoid as well as nontyphoid cases.

TABLE 5

PERCENTAGE COMPARATIVE TABLE OF OUR WEEKLY POSITIVE RESULTS WITH THOSE OF OTHER INVESTIGATORS

Weeks	Blood Culture		Complement Fixation, Author's Percentage	Agglutination	
	Coleman and Buxton	Author's Percentage		Author's Percentage	Park and Williams
First	89	94	68	50	20
Second	73	40	75	50	60
Third	60	23	92	60	89
Fourth	38	0	100	88	90
Fifth	26	0	100	70	75
Average*	69	45	80	55	65 ?

* The average percentages were computed on the total number of tests.

In table 5, despite the minor variations in the weekly findings, the slope of the corresponding curves is practically the same as in chart 1.

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[5] Am. Jour. Med. Sc., 1907, 133, p. 896.

[6] Path. Microorganisms, 1917, p. 345.

In blood cultures the peak is reached in the first week and in agglutinations during the fourth week, which means that blood culture is of minor value in the latter and agglutination in the early course of the disease.

The pathologic conditions giving rise to nonspecific complement fixation, with the exception of those in one case of typhus, clinically also could be differentiated from typhoid. On the other hand, nonspecific agglutination occurred almost 100% in acute infectious diseases such as typhus, malaria, miliary tuberculosis, acute mastoiditis, acute appendicitis and influenza, that could not be easily differentiated from typhoid without further clinical and laboratory data.

SUMMARY

In the course of typhoid infection, the formation of complement-fixing antibodies is one of the earliest and most constant immune manifestations.

The introduction of a differential quantitative, active serum technic for the complement-fixation test makes it possible to utilize this property for the diagnosis of typhoid fever, with satisfactory results.

In a limited number of typhoid cases the test has been repeated with inactive serum and found similarly satisfactory It is recommended, however, that the active serum technic be used, as it saves much time in the routine work of the test.